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ABSTRACTS FROM THE ORIGINAL PAPERS.

Studies on Proteins. I.

(Contribution No. 2. from the Laboratory of Nutritional Chemistry, Department
of Agriculture, Kyoto Imp. University)

By Kinsuke KONDO.

A. A brief account is given of some of the most important researches hitherto published on the occurence of combination between protein and mineral acid or base. (1) One view says that the binding of protein to mineral acid (strong acid as HCl) or alkali is not chemical combining in a true sense, but only absorption phenomenon. (2) The other view says that protein and acid or alkali really combine chemically. In the latter view, we can find two branches. (a) The compounds of protein with acid or alkali do not yield on electric dissociation the simple anion of acid or cation of base, in one or more of which the inorganic radical is bounded up in a non-dissociable form. T. B. Robertson holds this opinion. (b) The compounds of protein with acid or alkali can yield on dissociation the simple anion of acid or cation of base in question. W. Pauli, S. P. L. Sörensen, J. Loeb hold this view. Examining the experiments, the different views grounded on, the latter opinion is most probable,······the writer believes······regarded from the point of modern view of the matter.

B. The casein employed in my investigations was explained. It was prepared by Kahlbaum according to the Hammarsten method. The water content, determined partly by drying in vacuo for 24 hours at 80°C, partly by standing for three months in a sulphuric acid desiccator, was 9.43%. The ash content and nitrogen content of the dried casein were 0.15 % and 15.46% respectively. The method of nitrogen determination was explained briefly. The factor by which the quantity of nitrogen in the casein must be multiplied to give the corresponding weight of dried casein is thus 100/15.46 = 6.47.

C. The measurements of the solubility of casein in HCl revealed the peculiar fact that the solubility decreases……with the same concentration of HCl to begin with……as the quantity of precipitates in contact with the solution increased.

I have postponed the explanation of this feature until more comprehensive investigations have been made, but have just suggested that it should perhaps be sought in the capacity of casein to cause complex binding of chlorine ions both in the solution and together with hydrogen ions in the precipitate and solution being well calculated to explain the experimental results.

D. It was examined whether the casein in HCl–solution studied alters or not, through the determining of formol–titrable nitrogen. The results came to that the casein can keep the same nature in the solution of different concentration of HCl employed. It was found that the casein forming the casein chloride in HCl–solution occupies 0.891c.c. per 1g of dried casein.

E. In this section the writer has thrown one glance on the activity theory of ion.

(a) The theoretical considerations and experimental works were made for the determination of chlorine ion activity. The results came to the expression

$$pa_{Cl} = \frac{0.0674 - E_{Cl}}{0{,}0577} \quad \text{..........at } 18^{\circ}\text{C.} \cdots\cdots (1)$$

where $pa_{Cl} = -\log a_{Cl}$, and a_{Cl} is the chlorine ion activity in the solution and E_{Cl} is the potential of the following elements:

Hg, HgCl, 0.1n KCl | 3,5n KCl | Exp. liquid, HgCl, Hg.……………………(I)

The measurements were carried out in a thermostat at 18°C.

(b) For determination of the activity of the hydrogen ion I got the following formula through precisely the same theoretical considerations and experimental results as with the determination of chlorine ion activity:

$$pa_H = \frac{E_H - 0.3348}{0.0577 + 0.0002(t - 18^{\circ})} \quad \cdots\cdots (2)$$

where $pa_H = -\log a_H$, and a_H is the hydrogen ion activity in the solution and E_H the potential of the following element:

Hg, HgCl, 0.1n KCl | 3.5n KCl | Exp. liquid, H_2, Pt.……………………(II)

The measurements were made with the Hasselbach's shaking electrode in an air thermostat.

(c) By measurement of hydrogen ion activity and chlorine ion activity in the solutions of casein-hydrochloric acid, and reckoning back to concentrations of these two ions after the activity theory, it was found that the casein in the solution binds per g (not dried) about 2.10 gram equivalents of chlorine ions, and dissociation degree of casein chloride is about 0.72. It will be also noted that the dissociation degree of casein chloride and the number of complex-bound gram-equivalents of chlorine ions per g casein, appear to be independent of the hydrogen ion activities used.

(June 18, 1925.)

On Sulphur-containing Amino-acids. V.

On the Oxidation of Cystine and Cysteine by Iodine.

by YUZURU OKUDA and MUNEKUMA SAMEJIMA.

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Friedmann (Beitr. Chem. Physiol. Pathol., 3, 1. 1902) has found that cystine as well as cysteine are oxidized by bromine into cysteic acid, and Okuda (J. Tokyo Chem. Soc., 37, 181, 1916, and J. Chem. Soc. Japan, 45, 1, 1924.) studied this oxidation quantitatively, and ascertained that in these cases one molecule of cystine requires 10 atoms of bromine and one molecule of cysteine 6 atoms, and applied these facts for the determination of them.

Lately, Okuda (J. Chem. Soc. Japan, 45, 1, 1924 and 19, 1924.) also found that among amino acids cysteine alone reacts with iodine very actively in an acid solution and at room temperature, and applied this fact for the determination of cystine and cysteine. But on the oxidation products in this case no further investigation was performed.

In the present investigation the following facts were ascertained:-

1. At higher temperature both cystine and cysteine are oxidized into cysteic acid, one molecule of cystine requiring 10 atoms of iodine and one molecule of cysteine 6 atoms of iodine for oxidation. **2.** At room temperature and in hydrochloric acid solutine, cystine does not react with iodine. But cysteine does, and the chief oxidation product is cystine, accompanyed by a little cysteic acid. The ratio of the quantities of the oxydation products depends upon the acidity and temperature of the sample.

On the Application of Aging yeast (Willia anomala) to Saké and Saké artificial.

By Teizo TAKAHASHI.

(Faculty of Agriculture, Tokyo Imperial University.)

It was about fifteen years ago that the writer has reported on the nature of aging yeast of saké and its application to the saké manufacture. (Journ. of the Callege of Agric. Vol. I. No. 3. p. 227–268, 1911). At the time it was applied, in pure culture, in the begining of "moto"–mash or at the stage of "Moromi"–mash or at the aging stage of saké, i. e. after the pressing of "Moromi"–mash.

The aging effect was most conspicuous when it was discovered. Howerer, recently it happened that aging nature of this yeast to saké has almost been lost after too long series of successive generations in artificial cultare medium.

In 1921, the writer changed the medium to an incomplete one, devoid of carbohydrate which was replaced by ethylalcohol as shown below :–

Alcohol abs.	5 c.c.
Asparagine.	2.5 g.
$K\ H_2P_2O_4$.	1.0 g.
$MgSO_4$.	0.34 g.
Water.	95.0 c.c.

The culture in such a defective medium showed very good growth and gave favourable change for the ripening of young or unmatured saké. The change of chemical composition of saké durig aging by this yeast is shown below :–

	Saké, original.	Saké, aged.
P_H value.*	4.3	4.3
grams in 100 c.c.		
Extractive matters.	4.218	4.196
Total acids. as succinic acid.	0.215	0.223
Total nitrogen.	0.185	0.182
Protein matter. (Stutzer's method).	0.065	0.067
Dextrin	0.745	0.457
Reducing sugars.	1.48	1.248
Ethyl alcohol	17.24 vol %	17.64 vol %
Esters (as di-ethiyl succinate).	0.089	0.092

Colloid matters. Drum number.	1239	1206
Colloid number. •	66	45

* After L. Mihaelis' colorimetric method. (L. Mihaelis und. A. Gyemant. Bioch. Zeit. 109, 165. 1920.; L. Mihaelis und. A. Krüger. Bioch. Zeit. 119, 307. 1921.)

• Author's method. This journal vol. I. No. I.

The yeast do favours similary for the aging of artificial ones.

On the Refractometric Quantitative Analysis of Alcohol and the Extract in Sake.

By Mitsuji Ito.

Experiments on the refractometric quantitative determination of ethyl alcohol in spirituous liquor have been made by several authors, but so far its application for the analysis of *Sake*, a Japanese alcoholic beverage made of rice, has not been done.

In order to determine alcohol and the extract in *Sake* quantitatively by the use of Pulrich's refractometer, I have examined many kinds of *Sake* produced in various districts of Japan; and have found the following formulae for the calculation of alcohol and the extract indirectly. These resulting figures were compared with those obtained directly by the ordinary method.

I. For the calculation of alcohol in volume %.

$$X = \frac{R-(W+U\times E)}{D} \quad \cdots\cdots \text{Formula a.}$$

$$A' = X \pm x \cdots\cdots (1) \qquad U = \frac{r-r'}{e} \cdots\cdots (2)$$

Where, X = Calculated % of alcohol in volume.

A' = Required % of alcohol in volume.

x = Average supplemental number found from the examination of many kinds of *Sake*.

R = Refractive index of *Sake*, measured by the Pulfrich's refractometer at 20°C.

W = 1.33274, refractive index of water at 20°C.

E = Weight of extract, gram in 100 c.c. of *Sake*.

D = 0.0005, mean value of the refractive index corresponding to one volume % of alcohol at 20°C.

r = Mean value of the refractive index of *Sake*.

r′ = Refractive index of the alcohol solution, the concentration of which is equal to the mean value of that in *Sake*.

e = Mean value of the extrrct in 100c.c. of *Sake*, in gram.

U is calculated as follows:– 0.00192 is the mean value for 72 kinds of new and old *Sake*, 0.00190 for 59 kinds of old *Sake*. 0.00188 for 35 kinds of Honshū old *Sake*, 0.00189 for 31 kinds of Kwansai old *Sake*, 0.00193 for 24 kinds of Hokkaido old *Sake*, and 0.00203 for 13 kinds of Hokkaido new *Sake*. Using 0.00197 (mean value between 0.00190 and 0.00203) for the value of U, the above formula may be shown as follows.

$$X = \frac{R-(1.33274+0.00197\times E)}{0.0005} = \{R-(1.33274+0.00197\times E)\}\times 2000 \cdots$$

$$\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \text{Formula a}'.$$

II. For the calculation of the extract, gram in 100c.c.

$$X = \frac{R-(W+D\times A)}{U} \cdots\cdots\cdots\cdots\cdots\cdots \text{Formula b}.$$

$$E' = Y \pm y \cdots\cdots\cdots\cdots\cdots\cdots (3)$$

Where, E′ = Requred weight of the extract in 100c.c. of *Sake*.

y = Average supplemental number found from the examination of many kinds of *Sake*.

A = Volumetric % of alcohol in *Sake* measured by specific gravity of the distillate as usual.

R, W, D and U are the same as the above.

The above formula b may be shown as follows.

$$Y = \frac{R-(1.33274+0.0005\times A)}{0.00197} = \{R-(1.33274+0.0005\times A)\}\times 508 \cdots\cdots$$

$$\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \text{Formula b}'.$$

The values of X calculated for the many kinds of *Sake* by the formula above descrived are nearly consistent with those of A obtained by ordinary method, and the mean value of x between X and A is –0.24 % for Kwansai old *Sake*. 0.07 for Kwanto and Tohoku old *Sake*, 0.18 for Hokkaido old *Sake*, 0.61 for Hokkaido new *Sake*, and 0.06 for all these kinds of *Sake* mentioned above. A′ is calculated from these X and x above obtained.

In like manner values of Y are nearly consistent with those of E obtained by ordinary gravimetric way, and the mean value of founded between Y and E is –0.0546g. for Kwansai old *Sake*, –0.0013 for Kwanto and Tohoku old *Sake*, -0.0108 for Hokkaido old *Sake*, 0.1495 for Hokkaido new *Sake*; and –0.0002 for all these kinds of *Sake* above said. E′ is calculated from these Y and y above obtained.

From the above results, it may be stated that the practical use for the refractometric quantitative analysis of alcohol and the extract in *Sake* or other its analogous alcoholic beverage is to be applied. The difference between the values of alcohol obtained by the refractometric method and those by the ordinary method is rather smaller than the experimental error which occurs between the primary distillate and the redistillate.

Aldehyde in Connection with the Saké-brewing.

By Masakazu YAMADA.

Recent works of Neuberg, Neubauer, Kurono, Embden, Oppenheimer etc., have showed that aldehydes and ketones play an important rôle as the intermediate products in various phases of fermentation.

In the main fermentation of Saké-brewing aldehydes have also been observed everytime but little study was made on them.

The author studied them from the standpoint that they might have relation to the delicate flavour of saké and as well as to its keeping qualities.

The only aldehyde, obtained by steam distillation of saké was acetaldelyde. It was identified from the melting point of its *aldomedon*? of its paro–nitro–phenylhydrazone and also from the content of nitrogen of the latter. The content of acetaldehyde was estimated by Ripper's bisulphite method at each period of brewing and storage.

At the stage of "moto"—cultivation of Saké–Yeast—and "moromi"—mainfermentation of saké—the content of acetaldehyde was quite small and showed little variation. Fresh and old saké are easily distinguished by the estimation of the aldehyde content, for aldehyde increases gradually during the storage period, thus the average content being 0.0057 percent in the fresh saké in April and 0.01117 percent in the old in October.

From the antiseptic nature of the acetaldehyde, though not very strong it might be supposed that its quantity is in relation of the resistance of saké against "hiochi"-a putrefactive phenomenon of saké caused by some lactic and acetic acid producing bacteria. The content of the acetaldehyde has been, in practice, measured by simply warming a portion of saké at intervals of about a month during the storage period. This is called "nomikiri" In the putrefied saké only trace of acetaldehyde is to be met with. It is imaginable that the acctaldelyde will be oxidised into acetic acid in putrefaction process, because the acid content of putrefied saké is ordinarily higher than that of the healthy one. Endly, aldehyde has nothing to do with the quality of saké.

In "shōchū"—Japaese whisky prepared by destilling the saké-cake—the flavaur is accurately proportional to its aldehyde content.